University of Alberta

Reflexive and peripheral contributions to muscle contractions evoked by tetanic electrical nerve stimulation in humans

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the

requirements for the degree of *Master of Science*

Centre for Neuroscience

Edmonton, Alberta

Fall 2005

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Abstract

Neuromuscular electrical stimulation delivered at 100 Hz can induce contractions that are considered extra to stimulating motor axons. The purpose of this study was to determine whether H-reflexes contribute to such "extra" contractions. Extra plantarflexion and dorsiflexion contractions were evoked in relaxed neurologically-intact persons by stimulating tibial and common peroneal (CP) nerves, respectively: at 20 Hz for 2 s – 100 Hz for 2 s – 20 Hz for 3 s (1-ms pulses). Isometric ankle torque and amplitude of H-reflexes and M-waves were evaluated before and after the 100-Hz burst. Overall, extra plantarflexion was evoked with stimulation just above motor threshold and was associated with larger soleus H-reflexes, while small M-waves remained unchanged. Greater stimulation intensity was required for extra dorsiflexion, during which H-reflexes remained small and M-waves predominated. In conclusion, soleus H-reflexes contributed to extra plantarflexion, independent of peripheral changes; however, factors other that TA H-reflexes contributed to extra dorsiflexion: enlarged M-waves and potentiated muscle twitches were often implicated.

Acknowledgement

I would like to express my gratitude to my readily accessible advisor Dave Collins for his unwavering encouragement and constructive feedback throughout the entire process, to my undergraduate research supervisor Paul Zehr for opening the doors into academia, to my teaching mentor Brian Maraj for his many insights during our conversations over the years, and to the energetic Vivian Mushahwar for setting aside time to further my research experience and scientific understanding. Also, I would like to acknowledge the numerous friends and colleges for their support and knowledge in shaping my analytical thinking about the world and myself. And ultimately, I would like to thank my family: my parents Ewa and Zygmunt for nurturing the optimal circumstances for my growth and life-long learning, my sister Anna for her competitive spirit, and my life partner Alejandra for her enduring sense of adventure, inquisitiveness and joyfulness.

Table of Contents

CHAPTER 1

Introduction and review of literature **1**

Neuromuscular electrical stimulation for rehabilitation 1

Activating muscles by NMES and measuring electromyography 1

Differences between synaptic and artificial recruitment of motor units 2

Reversed recruitment order of motor units contributes to muscle fatigue 3

Evoking an H-reflex by stimulating sensory axons 3

Activating spinal motoneurons with electrical stimulation 4

Depression of H-reflexes during tetanic stimulation 5

Asynchronous motoneuron discharge evoked by tetanic stimulation 6

Extra contractions evoked by high-frequency NMES 6

Plateau potentials in human spinal motoneurons 7

Recovery of H-reflexes during tetanic stimulation 8

Post-tetanic potentiation of H-reflexes 8

Recovery of H-reflexes and extra contractions 9

Assessing H-reflexes independent of M-waves 9

Potential peripheral contributions to extra contractions 9

Summary and significance 11

References 12

CHAPTER 2

Paper **17**

Introduction 17

Methods 18

Subjects *18*

Protocol 19
Electromyography 19
Nerve stimulation 19
Induction of extra contractions 20
Data acquisition and analysis 21

Results 21

Tibial nerve stimulation 22
CP nerve stimulation 24
Lower intensity CP nerve stimulation 24
Higher intensity CP nerve stimulation 25

Discussion 26

Reflexive contribution to extra contractions 27
Peripheral contributions to extra contractions 30
Implications 32

Figures 34

Figure 1 34
Figure 2 35
Figure 3 36
Figure 4. 37
Figure 5 38
Figure 6 39
Figure 7 40

References 41

CHAPTER 3

Conclusion and general discussion **45**

Afterthoughts on reflexive contributions to extra plantarflexion 45
Afterthoughts on other than reflexive contributions to extra dorsiflexion 45
Revisiting extra contractions during proximal nerve block conditions 46
Experiments related to potentiated H-reflexes 48

References 50

List of Figures

Figure 1. Plantarflexion and EMG responses evoked by tibial nerve stimulation in a single subject. A: normalized isometric torque generated by the 20-100-20-Hz stimulation, and normalized amplitude of each H-reflex and M-wave during 20-Hz stimulation before and after the 100-Hz burst. Also shown are torque, H-reflexes, and M-waves evoked by 20-Hz stimulation for 7 s (see legend for responses in trains with and without the 100-Hz burst). Data are averaged from 5 trains. Stimulation pattern with the 100-Hz burst (black line) and without the burst (grey line) are represented beneath the plot. B: Electromyograms from a single 20-100-20-Hz stimulation train, and beneath on an expanded time scale. Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 3B (grey triangle).

Figure 2. Effectiveness of the 100-Hz burst for increasing amplitude of H-reflexes and torque, compared to 20-Hz stimulation without the burst at matched stimulation intensity. A: pooled results (N=3) demonstrating increased H-reflexes after the 100-Hz burst. Mean responses, before and after the burst, are represented for each subject in Fig. 3B (dark square, dark diamond and grey triangle). Torque (grey line) from 20-Hz stimulation alone is truncated at 7 s because stimulation was 10 s in one subject. B: pooled results (N=3) demonstrating ineffectiveness of the 100-Hz burst at further increasing H-reflexes, compared to intensity-matched stimulation without the 100-Hz burst. Mean responses for each subject are represented in Fig. 3B (grey square, white triangle and white circle).

Figure 3. Group results of tibial nerve stimulation at 20-100-20-Hz for 7 s. A: pooled results of 9 subjects (vertical lines represent 95% confidence interval). B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the stimulation train; amplitude of this response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line, as well as N.S. changes by dashed lines.

Figure 4. CP nerve stimulation at lower intensity in a single subject with extra dorsiflexion accompanied by increased TA M-waves and H-reflexes after the 100-Hz burst. Electromyograms are shown from a single train during 20-Hz stimulation, at onset (left) and after the 100-Hz burst (right). The amplitude of each M-wave was measurable apart from the stimulus artifact (truncated). Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 5B (grey triangle).

Figure 5. Group results of CP nerve stimulation at 20-100-20-Hz for 7 s at lower stimulation intensity (mean M-waves at $time_1$ were between 5-20% M_{max}). A: pooled results of 6 subjects (vertical lines represent 95% confidence interval. B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the train; amplitude of this response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line, as well as N.S. changes are denoted by dashed lines ($P=0.130$ and $P=0.074$ for pooled mean M-wave and H-reflex, respectively).

Figure 6. CP nerve stimulation at higher intensities in a single subject with extra dorsiflexion without sufficiently increased EMG responses after the 100-Hz burst. Extra torque was not associated with increased M-waves or H-reflexes, relative to EMG responses during 20-Hz stimulation alone. Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 7B (white circle).

Figure 7. Group results of CP nerve stimulation at 20-100-20-Hz for 7 s at higher stimulation intensity (mean M-waves at $time_1$ were between 35-65% M_{max}). A: pooled results of 7 subjects (vertical lines represent 95% confidence interval). B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the train; amplitude of the response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line and N.S. changes are denoted by dashed lines ($P=0.065$ and $P=0.128$ for pooled mean M-wave and H-reflex, respectively). Note: for reasons of scale, mean EMG responses are not shown for one subject (grey square): at $time_1$ and $time_2$ the mean M-wave was 3 and 4 ($P=0.18$), respectively, normalized to the first M-wave (16% M_{max}), and the mean H-reflex was 0.3 and 2.7 ($P=0.005$), respectively, normalized to the first H-reflex (0.7% M_{max}).

CHAPTER 1 – Introduction and review of literature

Neuromuscular electrical stimulation for rehabilitation

Neuromuscular electrical stimulation (NMES) is a tool for improving motor function in individuals with disability of the central nervous system (CNS), in particular in cases of stroke and spinal cord injury (SCI), and to some extent in cases of cerebral palsy and multiple sclerosis (Kilgore and Kirsch 2004; Popovic et al. 2001). NMES is commonly applied for therapy of atrophic changes related to disuse (therapeutic electrical stimulation) or for restoring specific functions such as mobility and motor control (functional electrical stimulation) (Stein et al. 2002). Essentially, NMES bypasses the damaged motor pathways of the CNS and provides the drive to produce contractions of otherwise paralyzed muscles. In diseases of the peripheral nervous system, however, NMES has not been readily implemented due to the difficulty in activating muscle tissue directly because muscle threshold exceeds levels for safe stimulation (Kilgore and Kirsch 2004; Salmons 2004). Over the long term, NMES induces changes that enable sustained work production by muscles: in persons with complete SCI, for instance, NMES applied for 6 weeks at 1 h/day has the capacity to reverse muscle fatigability, which is associated with muscle atrophy and slow- to fast-twitch muscle fiber transformation (from fatigue resistant to fast-fatigable fiber type) (Stein et al. 1992, 2002). Also, NMES prevents bone density lose and improves cardio-respiratory fitness by restoring muscle strength and overall mobility (Stein et al. 2002). In conjunction with conventional occupational and physical therapy, NMES remains the most promising approach in rehabilitating patients with damaged motor pathways of the CNS (Popovic et al. 2001).

Activating muscles by NMES and measuring electromyography

In the simplest form, NMES artificially depolarizes a segment of intact nerves by delivering pulses via surface (transcutanous) or implanted (percutanous) electrodes (Popovic et al. 2001). Once motor axons are artificially activated, propagation of action potentials along axons and release of neurotransmitter at the neuromuscular junction appear similar to events generated physiologically at the motoneurons (Salmons 2004; Baker et al. 2000). The branches of a single motor axon are normally in a "one-to-one" relationship with their muscle fibers, such that one motoneuron and the muscle fibers it innervates constitute a motor unit. Small motor units consist of fewer numbered and

more fatigue resistant muscle fibers, whereas large motor units consist of greater numbered and rapidly fatigable muscle fibers (Burke 1981). At the neuromuscular junction, the arrival of action potentials causes the neurotransmitter acetylcholine to be released. This initiates a local depolarization of the postsynaptic membrane, which in turn generates muscle action potentials in the surrounding sarcolemma that propagate along the length of the muscle fiber. Finally, through a sequence of events, involving Ca^{2+} release from the sarcoplasmic reticulum and interaction of proteins actin with myosin, a muscle contraction is evoked (Monnet and Salmons 2004). The compound muscle action potentials can be recorded at the target muscle by surface electromyography (EMG), such as those potentials that constitute the direct motor response evoked by stimulating motor axons: termed the M-wave.

Differences between synaptic and artificial recruitment of motor units

Artificial recruitment of motor units by stimulating motor axons and synaptic recruitment of motor units by physiological activation of motoneurons are different in at least one functional aspect: the recruitment order of motor units is generally reversed with NMES (Baker et al. 2000; Salmons 2004). Synaptic recruitment proceeds in accordance with the "size principle" by which small motoneurons reach threshold first (Henneman et al. 1965). Small motoneurons have the greatest input resistance, so their cell bodies experience the largest membrane depolarization for a given synaptic current (Enoka 2002). In the physiological order, thus, smaller motor units (fatigue resistant) are recruited first and larger motor units (rapidly fatigable) are recruited last (Burke 1981; Henneman et al. 1965). On the contrary, with NMES the recruitment order generally proceeds from larger to smaller motor units (Blair and Erlanger 1933; Fang and Mortimer 1991; Feiereisen et al. 1997; Salmons 2004; Solomonow 1984). Despite the general consensus that reversed recruitment order is fundamentally determined by axonal biophysical properties, other factors also influence recruitment order: such as stimulation method (nerve cuff or surface NMES; Knaflitz et al. 1990), the relative distance between axons and stimulation site and the organization of axonal branches (Feiereisen et al. 1997), and injury related changes in structure and organization (Enoka 2002; Thomas et al. 2002). Nonetheless, in the classical view, larger motor axons that innervate larger motor units are more readily excitable because of their low axial resistance for current flow —so larger axons experience the largest membrane depolarization for a given

transmembrane current (Enoka 2002). In practice, however, such a reversal of recruitment order is far from precise, thus, it may be more accurate to speak of disorderly recruitment or at the least of a tendency for reversal of recruitment order (Salmons 2004).

Reversed recruitment order of motor units contributes to muscle fatigue

A tendency for reversed recruitment of motor units is one factor that explains the excessive fatigability of muscle contractions evoked by NMES: because the least fatigue resistant muscle fibers are in constant use (Salmons 2004). Preferentially activating larger motor axons, which innervate fast-fatiguing, glycolytic muscle fibers, results in greater metabolic exhaustion of contractile mechanisms (Baker et al. 2000). Muscle fatigability due to the failure of contractile mechanisms, rather than failures in generating M-waves (muscle action potentials), can be demonstrated during NMES. In persons with SCI, for instance, the force of contracting muscles declined >60% while the size of M-waves was maintained during 3 minutes of intermittent 20-Hz stimulation (Stein et al. 1992). Even in able-bodied persons, force production declined by 20% during the same stimulation protocol (Stein et al. 1992). In addition to recruitment order, other factors contribute to the excessive fatigability of contractions evoked by NMES: such as synchronous activation of limited muscle fibers, high-frequency failure of muscle membrane excitability, restricted blood flow to contracting muscles, and disuse-related atrophic changes of muscles (Baker et al. 2000; Salmons 2004).

Evoking an H-reflex by stimulating sensory axons

Electrical stimulation of mixed peripheral nerves also activates sensory axons. In addition to an M-wave, a reflexive motor response —termed the H-reflex— can be evoked by stimulating group Ia afferents, which innervate muscle spindle receptors. First described by Hoffmann (1918), the H-reflex is considered the electrical analogue of the stretch reflex, but unlike its mechanical counterpart the electrically-evoked response bypasses muscle stretch receptors and is induced by a more synchronous and a differently assorted afferent volley (Burke and Schiller 1976). Compared to an M-wave, the H-reflex is a relatively longer-latency motor response since the reflex arc extends from the stimulation site to the spinal cord, at which point motoneurons are synaptically recruited, then back to the target muscle. With peripheral nerve stimulation, an H-reflex can be observed without an M-wave because larger diameter axons of Ia afferents are generally

recruited before smaller diameter motor axons (Zehr 2002). The largest H-reflexes are evoked with stimulus pulses between 0.5 and 1 ms because sensory rather than motor axons are preferentially activated, due to differences in excitability in accordance with the axon diameter (Veale et al. 1973, Panizza et al. 1989; 1998); yet even wider pulses (2 or 3 ms) have been shown to inhibit the H-reflex, possibly due to a relative increased activation of cutaneous axons. With narrower pulses (<0.3 ms) relatively more motor axons are excitable than sensory axons (Panizza et al. 1989; 1998). The H-reflex and M-wave do not consist of the same motor units: large diameter axons, innervating larger motor units, are first involved in the M-wave involves and smaller motor units are first recruited in the H-reflex (see Activating spinal motoneurons with electrical stimulation). At low stimulation intensity the reflex appears in the EMG produced by small motor units, in which circumstance an antidromic collision with motor axons has not taken place. With progressively increasing stimulation intensities, however, a reflex generated by larger motor units is subject to collision with antidromic volleys in motor axons. Nonetheless, this limit in H-reflex size cannot be completely explained by antidromic collision, since it occurs even at intensities below motor threshold. There is at least 1 ms between the first and last individual motoneurons contributing to the H-reflex, thus, an inhibitory disynaptic inputs may additionally limit the reflex size. Such inputs, which reach the motoneurons about 0.8 ms after the Ia volley, consist of Ib inhibitory interneurons that are co-activated by Ia afferents and Renshaw cells activated by the first motoneuron to discharge in the H-reflex (Pierrot-Deseillingly and Mazevet 2000). In the first part of the H-reflex (0.6 ms), the monosynaptic Ia excitation is probably not contaminated by any non-synaptic effects, yet because the induced afferent volley likely consists of a wide variety of afferents traversing non-monosynaptic pathways (Burke 1983), the reflex size can be potentially modified by changes in transmission through interneuron. However, various conditioning stimulation of group Ib or II afferents have demonstrated that the effects on the reflex size are insignificant compared to changes in excitability of motoneuron and/or presynaptic inhibition of Ia terminals (Pierrot-Deseillingly and Mazevet 2000).

Activating spinal motoneurons with electrical stimulation

One novel approach that may circumvent recruitment reversal with NMES is intraspinal microstimulation (ISMS), delivered through microwires implanted in selected

spinal cord segments (Mushahwar et al. 2000). Preliminary results of ISMS in rats demonstrate that prolonged ISMS at 20 Hz may be capable of progressively recruiting skeletal muscle motor units from smallest to largest sizes, as a significant mixed population of fiber-types (including fatigue resistant) were demonstrated to be activated (Bamford et al. 2003, 2004). Matched stimulation of peripheral nerves resulted mainly in activation of fast-fatigable fibers, which was in agreement with the tendency for reversal recruitment order with NMES. Outcomes of ISMS were explained by either indirect activation of motoneurons via reflex pathways, because afferents were more likely activated at lower thresholds than motoneurons, or by direct activation of motoneurons at the highest stimulation intensities (Bamford et al. 2003; Mushahwar et al. 2003).

Alternatively, a less invasive method of synaptically recruiting spinal motoneurons is via stimulating nerve afferents—as in evoking an H-reflex. Such a reflexive recruitment of motor units is generally suggested to proceed according to the "size principle" (Awiszus and Feistner 1993; Buchthal and Schmalbruch 1970; Desmedt and Godaux 1978; Henneman et al. 1965; Somjen et al. 1965; Taborikova and Sax 1968; Zehr 2002). Although not conclusive, the evidence suggests that smaller motor units (fatigue resistant) are recruited preferentially in many muscles (most notably in the soleus) when stimulation is applied to evoke H-reflexes (Awiszus and Feistner 1993; Buchthal and Schmalbrunch 1970). Despite this potential for orderly recruitment of motor units, H-reflexes are generally depressed during tetanic stimulation.

Depression of H-reflexes during tetanic stimulation

Although H-reflexes can be evoked in many muscles, the amplitude of reflex responses declines rapidly during repetitive stimulation. H-reflexes are well known to be significantly depressed for several seconds following a previous activation of the reflex arc (Burke and Schiller 1976; Crone and Nielsen 1989; Hultborn et al. 1996; Taborikova and Sax 1969). This post-activation depression is probably due to reduced neurotransmitter release from previously activated Ia afferents (Crone and Nielsen 1989; Hultborn et al 1996). Larger H-reflexes, may overcome some of the depressive effects with repeated activation, however, the extent of depression of spinal reflex responses is directly related to the frequency of stimulation (Lloyd et al. 1957a, b; Schindler-Ivens and Shields 2000; Van Boxtel 1986). Even substantially larger H-reflexes, those involving 20-50% of the motoneural pool, are subject to extensive depression during repetitive

stimulation. For instance, relative to the amplitude of H-reflexes evoked at 0.1 Hz, those evoked at 1, 5, 10 Hz are typically depressed by more than 65%, 75% and 90%, respectively (Schindler-Ivens and Shields 2000). With frequencies 25 to 100 Hz, H-reflexes are typically completely depressed (Burke and Schiller 1976). Since generating a continuous muscle contraction by NMES requires frequencies between 10 to 50 Hz, H-reflexes are therefore conceded to be absent and the resulting muscle contractions are generated by stimulating motor axons alone (Baker et al. 2000; Salmons 2004).

Asynchronous motoneuron discharge evoked by tetanic stimulation

Low intensity NMES at higher frequencies (25-100 Hz) can induce discharges of spinal motoneurons via activating group Ia afferents (Burke and Schiller 1976; de Gail et al. 1966; Lang and Vallbo 1967). Even though H-reflexes were absent, asynchronous motoneuron discharge emerged that was not time-locked to the driving stimulus. For instance, during the low-intensity tetanic stimulation of tibial nerve afferents, a complete depression of H-reflexes after the first H-reflex was reported; and after a ~10-s silent period, tonic EMG activity and contractions developed (Burke and Schiller 1976). However, during tonic vibration of muscles and tendons at 25-200 Hz, motoneuron discharge was demonstrated to be synchronized to the vibration cycle (de Gail et al. 1966; Lang and Vallbo 1967; Burke and Schiller 1976). This paradoxical ability to induce synchronized motoneuron discharge by high-frequency vibration but not by high-frequency electrical stimulation was in part explained by the size of afferent volley induced by the powerful vibration compared to low intensity electrical stimulation, in addition to differences in afferents involved and central transmission (Burke and Schiller 1976; Van Boxtel 1986). Thus, NMES at low intensity can evoke muscle contractions due to asynchronous motoneuron discharge yet transmission via the H-reflex pathway remains depressed.

Extra contractions evoked by high-frequency NMES

NMES consisting of 1-ms pulses delivered at 100 Hz over the muscle belly of either ankle plantarflexor or dorsiflexor muscles can induce strong contractions that are thought to be extra to those due to stimulating motor axons directly (Collins et al. 2001, 2002). For instance, during a stimulation train at 25-100-25 Hz, the muscle force was bigger after a 2-s 100-Hz "burst" than before it (Collins et al. 2001). The following

evidence suggests that such "extra" contractions are spinal of origin: these can be evoked at stimulation intensities below motor threshold and are often sustained after the stimulation ends by self-sustained spinal motoneuron discharge (Collins et al. 2001, 2002), which was shown to be unaccounted for by changes in excitability at the cortical and muscular levels (Nozaki et al. 2003). Remarkably, extra contractions are absent during anesthetic nerve block proximal to the stimulation site (Collins et al. 2001, 2002), and are present in persons with a clinically complete SCI (Nickolls et al. 2004). Thus, the evidence indicates that activated spinal motoneurons contribute to extra contractions.

Although extra contractions may involve activation of spinal motoneurons, H-reflexes were presumed to be absent (Collins et al. 2001, 2002; Nickolls et al. 2004) because these are know to be completely depressed during tetanic stimulation (Burke and Schiller 1976; Schindler-Ivens and Shields 2000). Instead, extra contractions were proposed to be due to asynchronous motoneuron discharge not time-locked to the stimulus (Collins et al. 2001, 2002; Nickolls et al. 2004), similar to that demonstrated during low-intensity tetanic stimulation of tibial nerve afferents in the absence of H-reflexes (Burke and Schiller 1976; Lang and Vallbo 1967). Furthermore, Collins et al. (2001) proposed that extra contractions are due to non-classical behaviours of spinal neurons: once motoneurons were recruited by the high-frequency afferent input, their discharge was sustained by intrinsic membrane depolarization at their preferred firing rate, in which circumstance motor unit firing was decoupled from the stimulus pulses. Thus, extra contractions were explained by the activation of plateau potentials within spinal neurons (Collins et al. 2001).

Plateau potentials in human spinal motoneurons

Studies using reduced animal preparations have demonstrated that intrinsic properties of motoneurons generate sustained membrane depolarization (i.e., plateau potentials), thereby sustaining the motoneuron discharge (Hultborn 1999). Several studies report the emergence of plateau potentials in humans (Gorassini et al. 1998, 2002; Collins et al. 2001, 2002; Kiehn and Eken 1997; Nozaki et al. 2003; Nickolls et al. 2004); however, due to experimental limitations the evidence at best remains indirect. Nonetheless, it is thought that plateau potentials can dramatically alter synaptic inputs and contribute to motoneuron discharge (Lee and Heckman 2000; Powers and Binder 2000). For instance, plateau potentials in a motoneuron may account for about 40% of

the required current by its cell body to discharge action potentials, as demonstrated using a paired motor unit method in humans (Gorassini et al. 2002). Also self-sustained motoneuron discharge that outlasted a 2-s period of tetanic stimulation was attributed to activating plateau potentials in spinal neurons, since the EMG activity was not fully accounted for by changes in excitability at the cortical and muscular levels (Nozaki et al. 2003). In terms of force output, discharge of spinal motoneurons sustained via activation of plateau potentials has been suggested to generate large contractions (up to ~40% of maximal voluntary activation) during high-frequency NMES (Collins et al. 2001, 2002).

Recovery of H-reflexes during tetanic stimulation

Activating plateau potentials in human spinal neurons has also been attributed to underlie the unconventional H-reflex behaviour during tetanic stimulation: during 50-Hz stimulation of tibial nerve afferents, H-reflexes gradually recovered from a complete post-activation depression, and returned to 20% of the initial value by the end of a 2-s stimulation period (Nozaki et al. 2003). Such rise in amplitude of H-reflex was analogous to the acceleration of motoneuron discharge at the initiation of plateau potentials (Nozaki et al 2003; Hultborn 1999). This effect always preceded the appearance of self-sustained discharge of spinal neurons after the stimulation ended (Nozaki et al. 2003). Alternatively, such unconventional H-reflex behaviour may have been due to temporal summation of post-synaptic potentials that occur with evoked spinal reflexes at frequencies above 60 Hz (Lloyd et al. 1957a, b); or was possibly due to potentiation of H-reflexes as classically seen after tetanic stimulation (Van Boxtel 1986).

Post-tetanic potentiation of H-reflexes

Tetanic stimulation of group Ia afferent fibers can result in post-tetanic potentiation (PTP) of H-reflexes both in animals (Curtis and Eccles 1960; Lloyd 1949; Wolpaw et al. 1989) and in humans (Hagbarth 1962; Van Boxtel 1986; Kitago et al. 2004). PTP can last for several minutes and is classically explained by mechanisms at the group Ia afferent terminal: probably resulting from increased mobilization and liberation of neurotransmitter (Curtis and Eccles 1960; Lloyd 1949). For example, after tetanic stimulation of the tibial nerve at 200 Hz for 20 sec, the amplitude of H-reflex evoked by single pulses at 1 Hz was significantly larger than before the stimulation. Such effect lasted more than 4 minutes, while the amplitude of M-waves was not

increased (Van Boxtel 1986). The stimulation periods used to induce PTP of H-reflexes range from tens of seconds to tens of minutes (Van Boxtel 1986; Kitago et al. 2004).

Recovery of H-reflexes and extra contractions

A recovery of H-reflexes, the like of which was recently demonstrated during tetanic stimulation of tibial nerve afferents (Nozaki et al. 2003), could potentially contribute to extra contractions. Thus, the purpose of the present investigation was to generate large extra contractions and to quantify changes in H-reflex and M-wave amplitudes during the stimulation period. To generate extra contractions, previously established optimal stimulation parameters (1 ms stimulus pulses at 100 Hz; Collins et al. 2001, 2002) were intermittently applied during stimulation trains at 20 Hz. Changes in the size of spinal reflex (H-reflex) were hypothesized to be associated with extra muscle activation. M-waves were expected to remain unchanged since peripheral changes are not expected to contribute because of the reported absence of extra contractions during anesthetic nerve block proximal to the stimulation site (Collins et al. 2001).

Assessing H-reflexes independent of M-waves

The size of the M-wave is typically measured as a means of monitoring stimulus consistency and peripheral excitability. To safeguard against methodological error, due to relative movement of stimulating electrodes or nerve, and to eliminate effects due to changes at peripheral sites, the H-reflex is typically evoked at intensities of stimulation that also evoke an M-wave (Zehr 2002). The novel finding of recovered H-reflexes during tetanic stimulation, however, was demonstrated at below motor threshold (Nozaki et al. 2003), and so an M-wave was not monitored.

Potential peripheral contributions to extra contractions

Peripheral changes were not expected to contribute to extra contractions because such contractions were abolished during nerve block conditions, presumably when such contributions could have manifested —but did not. Nonetheless, two peripheral mechanisms have been demonstrated to increase muscle force production, especially if a large proportion of motor axons is activated. Firstly, potentiation of M-waves has been demonstrated during high-frequency stimulation (100 Hz for 2 s) with nearly maximal activation of motor axons (McComas et al. 1994). Such potentiated M-waves likely

reflect changes in excitation of the muscle fiber membrane, probably due to an enhancement of electrogenic Na^+-K^+ active-transport mechanism in the muscle fibers' membrane (Hicks and McComas 1989; Cupido et al. 1996; McComas et al. 1994). Norepinephrine, released from intramuscular sympathetic nerve fibers, is one probable stimulus to increase the electrogenic capacity of Na^+-K^+ active-transport mechanism (Kuiack and McComas 1992; McComas et al. 1994), thereby leading to potentiated M- while the stimulation intensity remains unchanged. The resulting larger M-waves could potentially increase Ca^{2+} release, and in turn affect Ca^{2+} -dependent excitation-contraction coupling, thus, translating to increased force production (Klass et al. 1994; O'Leary et al. 1997).

Secondly, changes beyond the muscle membrane, independent of the amplitude of the M-wave, have been demonstrated after tetanic stimulation (100 Hz for 7 s), also at maximal intensities (involved maximal M-waves). The post-tetanic muscle twitches were larger, without immediate changes in amplitude of M-waves. Potentiated twitches have been attributed to enhanced excitation-contraction coupling and/or myosin-actin interaction (O'Leary et al. 1997; Vandervoort and McComas 1983). Such potentiation is directly related to the intensity, frequency and duration of stimulation (Brown and von Euler, 1938; Grange et al. 1993).

Furthermore, the extent of potentiation either of M-waves or of muscle twitches depends on the muscle's characteristics: because muscles with the highest proportions of fast-twitch fibers exhibit more potentiation (Belanger et al. 1983; Brown and von Euler, 1938; McComas et al. 1994; O'Leary et al. 1997). Although TA has a higher incidence of fast-twitch fibers and soleus has a higher incidence of slow-twitch fibers (Belanger et al. 1983), peripheral changes were not expected to contribute in to extra contractions of either ankle plantarflexors or dorsiflexors for several reasons: first, plantarflexors as a whole develop only slight potentiation that is largely determined by the potentiating capacity of soleus, which is virtually absent (Vandervoort and McComas 1983); second, extra contractions and a recovery of H-reflexes occurred at low intensities of stimulation, in which case no motor axons were activated (Nozaki et al. 2003); third, and most definitive, extra contractions were absent during anesthetic block of the nerve proximal to the stimulation site (Collins et al. 2001, 2002). Thus, peripheral contributions were not considered to be involved in extra contractions.

Summary and significance

The present study will determine whether increased H-reflexes, independent of peripheral changes (M-waves), contribute to extra muscle contractions evoked by high-frequency NMES in relaxed neurologically-intact humans (Collins et al. 2001, 2002). The soleus H-reflex will be investigated because it is known to recover during tetanic stimulation of tibial nerve afferents, and as such is likely it is hypothesized to contribute to extra contractions prevalent of ankle plantarflexors. While TA H-reflexes are less prevalent than soleus H-reflexes, the reflexive contribution to the apparently similar extra contractions of dorsiflexors will also be evaluated.

This novel stimulation paradigm, involving high-frequency stimulation bursts, may facilitate spinal reflexive activation of motoneurons, and thereby generate substantial contractions that are extra to those due to stimulating motor axons directly (Collins et al. 2001). Furthermore, such reflexive recruitment may circumvent the problems of muscle fatigue associated with the tendency for reversed recruitment order of motor units that occurs with conventional NMES.

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CHAPTER 2 – Paper

Introduction

While tetanic electrical stimulation of peripheral nerves in humans evokes muscle contractions via depolarizing motor axons, it can also affect the excitability of motoneurons via activating afferents (Burke and Schiller 1976; Collins et al. 2001, 2002; Lang and Vallbo 1967; Kido Thompson and Stein 2004; Knash et al. 2003; Khaslavskaia et al. 2002; Nickolls et al. 2004; Nozaki et al. 2003). In particular, neuromuscular stimulation delivered at 100 Hz over ankle plantarflexors and dorsiflexors can induce strong contractions that are considered extra to those due to stimulating motor axons directly: for instance, during stimulation at 25-100-25 Hz, the generated muscle force was larger after a 2-s 100-Hz "burst" than before it (Collins et al. 2001, 2002). Such "extra" contractions after the 100-Hz burst are inferred to be spinal of origin: these can develop at stimulation intensities below motor threshold and are often sustained after stimulation ceases (Collins et al. 2001, 2002) by self-sustained motoneuron discharge not associated with changes in excitability at the cortical and muscular levels (Nozaki et al. 2003), resembling the discharge after tendon vibration (de Gail et al. 1966; Lang and Vallbo 1967). Extra contractions were shown to be absent during anesthetic nerve block proximal to the stimulation site (Collins et al. 2001, 2002), yet were present in persons with clinically complete spinal cord injury (SCI) (Nickolls et al. 2004). Thus, evidence indicates a central mechanism involving the activation of spinal motoneurons contributes to extra contractions.

Although extra contractions probably involve a spinal mechanism, conventional H-reflexes were presumed to be negligible (Collins et al. 2001, 2002; Nickolls et al. 2004). These reflexes are powerful, such that a soleus H-reflex can involve between a quarter to all of the motoneural pool (Taborikova 1968), nonetheless, their amplitude is

well known to be depressed during tetanic stimulation (Burke and Schiller 1976; Crone and Nielsen 1989; Schindler-Ivens and Shields 2000; Taborikova and Sax 1968; Van Boxtel 1986). The extent that spinal reflexes are depressed is directly related to the stimulation frequency (Lloyd et al. 1957a,b; Schindler-Ivens and Shields 2000; Van Boxtel 1986): such that relative to H-reflexes at evoked at 0.1 Hz, those at 1, 5, 10 Hz are depressed by more than 65%, 75%, and 90%, respectively (Schindler-Ivens and Shields 2000); at 25 to 100 Hz, H-reflexes were shown to be completely depressed (Burke and Schiller 1976). This post-activation depression is likely due to reduced neurotransmitter release from previously activated Ia afferents onto spinal motoneurons (Hultborn et al. 1996). Instead of a reflexive contribution, extra contractions were proposed to be due to "asynchronous" motoneuron discharge, not time-locked to the stimulus, generated by the activation of plateau potentials in spinal motoneurons (Collins et al. 2001, 2002; Nickolls et al. 2004). Indeed, such asynchronous motoneuron discharge has been demonstrated during low-intensity tetanic stimulation of tibial nerve afferents in the absence of H-reflexes (Burke and Schiller 1976; Collins et al. 2001; Lang and Vallbo 1967). However, a partial recovery of H-reflexes, the like of which has been recently reported during 2 s of tetanic stimulation (Nozaki et al 2003), could potentially contribute to extra contractions. The present study was undertaken to bring together two presumably related findings: that extra contractions after a 100-Hz burst probably involve an activation of spinal motoneurons (Collins et al. 2001, 2002) and that H-reflexes recover during 50-Hz stimulation of tibial nerve afferents (Nozaki et al. 2003). However, H-reflexes were not investigated in the former and neither peripheral changes as reflected by M-waves nor muscle forces was monitored in the latter.

The purpose of this study was to determine whether changes in amplitude of H-reflexes contribute to extra contractions, free of peripheral changes. Soleus H-reflexes

were investigated because of their recently demonstrated recovery during tetanic stimulation (Nozaki et al. 2003) and as such were hypothesized to contribute to extra contractions prevalent in ankle plantarflexors (Collins et al. 2001, 2002; Nickolls et al. 2004). H-reflexes are not nearly as predominant in the relaxed tibialis anterior (Schieppati 1987; Zehr 2002), however, TA H-reflexes were investigated since extra contractions of ankle dorsiflexors are readily evoked by neuromuscular stimulation (Collins et al. 2001, 2002). M-waves were monitored to evaluate consistency of both stimulus and peripheral excitability (Zehr 2002). Parts of these data have been presented in abstract form (Klakowicz et al. 2004, 2005).

Methods

Subjects

Sixteen neurologically-intact persons (21-42 yr old; 11 males and 5 females) participated. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Health Research Ethics Board at the University of Alberta. In total, 11 and 13 subjects received stimulation to tibial and common peroneal (CP) nerves, respectively; there were 8 subjects that participated in both experiments on separate occasions.

Protocol

Only one muscle (soleus or TA) was studied per experimental session, which lasted between 1 to 2 hours. During the each experiment, the subject was seated with hip, knee and ankle at approximately 90, 110, 90°, respectively. Both feet were supported, and the right foot was strapped to a footplate configured to record isometric ankle torque (S-type load cell: LCCB-500; Omega, Stamford, CT). The trunk and right thigh were secured to minimize movement.

Depending on the muscle tested (soleus or TA), first the peak torque level over ~1 s was measured during a (3 to 5 s) maximal voluntary contraction (MVC) of ankle plantarflexors or dorsiflexors. The maximal peak-to-peak M-wave amplitude to a supramaximal pulse (M_{max}) was obtained from the relaxed muscle. Transcutanous tetanic electrical stimulation was delivered to the tibial or CP nerve to evoke extra contractions (see below). During the stimulation subjects were instructed to relax and not to contribute to the evoked contraction. They were encouraged to attend to other tasks (e.g., read or to listen to music). Isometric ankle torque and electromyographic activity (EMG) were recorded. The mean amplitudes of torque, M-waves and H-reflexes were quantified before and after a 2-s 100-Hz stimulation burst.

Electromyography

Surface EMG was recorded from the right soleus and TA, with bipolar (2.25cm^2) Ag-AgCl electrodes (Vermed Medical Inc., Bellows Falls, VT). EMG signals were preamplified 200-500× and band-pass filtered 10-1000 Hz (AMT-8, Bortec Biomedical Ltd, Calgary, AB).

Nerve stimulation

The right tibial and CP nerve was stimulated in the popliteal fossa and at the caput fibulae, respectively, at the most comfortable site that evoked a motor response (M-wave or H-reflex) at the lowest stimulation intensity. Often CP nerve stimulation activated ankle everters, resulting also in slight plantarflexion. The stimulation site was readjusted to preferentially evoke TA only, as determined by palpating muscles and monitoring ankle torque. The stimulation, consisting of 1-ms rectangular pulses, was delivered via bipolar surface electrodes from a Grass S88 stimulator (Grass Instruments, AstroMed, West Warwick, RI) connected in series with a CCU1 constant current unit and a SIU5 insulator unit. Stimulation current was measured (mA-2000 Noncontact Milliammeter, Bell Technologies, Orlando, FL).

Induction of extra contractions

Extra contractions were evoked using a stimulation patterned train adapted from a previous study (Collins et al. 2001): 20 Hz for 2 s – 100 Hz (burst) for 2 s – 20 Hz for 3 s (i.e., 20-100-20 Hz for 7 s). Five 20-100-20-Hz stimulation trains, 10 s apart, were included in each trial. Extra contractions were considered to occur if the mean ankle torque generated by 20-Hz stimulation was larger after the 2-s 100-Hz burst ($time_2$=5 to 6 s) than before it ($time_1$=1 to 2 s). Stimulation intensity was initially set at motor threshold, generally evoking a small M-wave and a nearly maximal H-reflex. During each trial, the stimulation intensity (current) was constant. If extra contractions were not observed, through visual inspection of torque during the trial, at the prearranged stimulation intensity, then a new trial was initiated and the intensity was progressively increased. Extra contractions were considered absent if the stimulation was painful, at which point the experimental session was terminated, or if the torque was not changed. The presence of extra contractions in each trial was confirmed after the experiment by statistical evaluation of the increase in torque after the 100-Hz burst, i.e., extra contractions were considered to occur only if the torque was significantly increased. For each subject, anywhere from 1 to 3 and from 3 to 6 trials were collected during stimulation of tibial and CP nerves, respectively, in each experimental session. Also, trials at matched intensity involving 5 trains at 20 Hz for 7 s (no 100-Hz burst) were collected in 6 and 4 subjects during tibial and CP nerve stimulation, respectively. The effective stimulation intensity for all trials is reported as the size of the mean M-wave at $time_1$ (averaged from 1 to 2 s, i.e., just prior to the 100-Hz burst), at which point consecutive M-waves were most steady.

Data acquisition and analysis

Data were sampled at 2 kHz using a custom-written program (LabView, National Instruments) and stored on computer for analysis. Torque was normalized to the MVC value. The amplitude of each M-wave and H-reflex during periods of 20-Hz stimulation was measured peak-to-peak and normalized to M_{max}. During 100-Hz stimulation EMG responses were not evaluated since the signal was typically contaminated by the stimulus artifact. For each subject data were averaged from the 5 successive stimulation trains in a trial. The mean size of EMG responses and torque during 20-Hz stimulation were

compared before ($time_1$=1 to 2 s) and after the 100-Hz burst ($time_2$=5 to 6 s); i.e., mean values from 1 to 2 s into the initial 20-Hz stimulation were compared to mean values at the same time into the following 20-Hz stimulation, after the 100-Hz burst. Data for individual subjects and the group are reported as mean (SD); 95% confidence intervals are shown for group data. For individual and group data, paired t-tests were used to assess significant differences between mean values from $time_1$ to $time_2$ (from before to after the 100-Hz burst). To identify significant differences between trials with and without the 100-Hz burst, mean values were compared at $time_2$, given that those at $time_1$ were matched. An α level of 0.05 was used to evaluate statistical significance.

Results

Both tetanic stimulation of the tibial and the CP nerves produced extra contractions: the contracting muscles during 20-Hz stimulation generated significantly larger isometric ankle torque after a 2-s 100-Hz burst (at $time_2$) than before it (at $time_1$). However, the extent to which H-reflexes contributed to extra plantarflexion and dorsiflexion was drastically different: H-reflexes contributed predominantly during tibial nerve stimulation, but not during CP nerve stimulation.

In total, extra contractions were generated in 9 of 11 and 10 of 13 subjects during stimulating tibial and CP nerves, respectively. Extra contractions did not develop in 2 and 3 subjects during tibial and CP nerve stimulation, respectively, which includes 1 terminated experiment in each case because of stimulating discomfort. Only results that demonstrated extra contractions were included for analysis because the present objective was to investigate the contribution of H-reflexes to these contractions.

Tibial nerve stimulation

The intensity of tibial nerve stimulation required to evoke extra contractions of plantarflexors was just above motor threshold: the mean M-wave amplitude at $time_1$ ranged 0.3 to 4% M_{max} among the subjects.

Results for an individual who exhibited immediately depressed H-reflexes from the first response, and then increased H-reflexes contributing to extra plantarflexion after the 100-Hz burst are shown in Figure 1. The peak-to-peak amplitude of each H-reflex and M-wave during 20-Hz stimulation and torque generated by the 20-100-20-Hz stimulation are represented (Fig. 1A). Also shown are amplitude of H-reflexes, M-waves and torque evoked by the 20-Hz stimulation for 7 s (no 100-Hz burst). Data are average of 5 successive stimulation trains. EMG activity from a single 20-100-20-Hz stimulation train (Fig. 1B) is shown for the entire stimulation period and beneath on an expanded time scale at stimulation onset (left) and after the 100-Hz burst (right). The first H-reflex was largest, measuring 34% M_{max} (SD 8) (pointed out by arrow in Fig. 1A), and the initial upward deflection in torque was ~4% MVC. Following the first response, H-reflexes were immediately depressed and remained so during the initial 20-Hz stimulation. From $time_1$ to $time_2$ (from before to after the 100-Hz burst), the mean H-reflex tripled from 3% M_{max} (0.4) to 9% M_{max} (0.8) ($P<0.001$), while the relatively smaller mean M-wave was elevated from 0.6% M_{max} (0.1) to 0.9% M_{max} (0.1) ($P<0.01$). The corresponding torque more than tripled from 3% MVC (0.4) to 10% MVC (0.8) ($P<0.001$). During 20-Hz stimulation for 7 s (no 100-Hz burst), the mean H-reflex and torque also gradually increased ($P=0.01$ and $P=0.02$, respectively) but not as much as after the 100-Hz burst. In the trials with the 100-Hz burst, the mean H-reflex at $time_2$ was 2-times larger ($P<0.01$) and the torque was nearly 3-times larger ($P<0.001$) than in the intensity-matched trial without the burst.

In most subjects (7 of 9) the extra torque was accompanied by increased H-reflexes; however, in 2 subjects small extra contractions were not accompanied by significant changes in H-reflexes and M-waves. To evaluate the effect of the 100-Hz burst, 6 trials with and without the burst were compared at matched stimulation

CP nerve stimulation

In general the intensities for CP nerve stimulation, as reflected by the mean M-wave at $time_1$, required to generate extra dorsiflexion contractions varied between subjects and was typically much higher (5-100% M_{max}) than for tibial nerve stimulation (<5% M_{max}). Therefore for comparison purposes data were grouped according to intensity of the CP nerve stimulation required for extra dorsiflexion contractions: at "lower" intensity the mean M-waves ranged 5-20% M_{max}, whereas at "higher" intensity the mean M-waves ranged 35-65% M_{max}. Of the 10 subjects who exhibited extra dorsiflexion contractions, these were evident in 6 subjects at lower intensities (in 2 subjects evoked mean M-waves of <10% M_{max}). Extra contractions were evident in a total of 7 subjects at higher intensities. Five of these subjects exhibited extra contractions at both lower and higher intensities and so were included into both groups. In the remaining 2 subjects, to generate extra dorsiflexion required maximal stimulation intensities, thus, evoking maximal M-waves and very strong contractions (~50% MVC); even though the stimulation was not reported to be painful, these results were excluded from further comparison because of the large difference in intensity required to produce extra dorsiflexion, compared to the other grouped subjects (mean M-wave of 7 and 50 % M_{max} at lower and higher intensity, respectively).

Although the effective stimulation intensity was gauged by the amplitude of the M-waves, changes in size of M-waves after the 100-Hz burst were not due to changes in actually stimulation intensity (current) because measured stimulation current remained constant throughout. Nonetheless, the mean M-wave at $time_1$ was used as an estimate of the actual stimulation intensity, which was constant from before to after the 100-Hz burst.

Lower intensity CP nerve stimulation

The effect of stimulating the CP nerve at lower intensity (mean M-wave of <20% M_{max}) are shown in Figure 4 for an individual who demonstrated extra dorsiflexion accompanied by small yet increased H-reflexes after the 100-Hz burst; however, M-waves also increased after the burst and were at least 10-times larger than H-reflexes. EMG activity demonstrates that peak-to-peak amplitude of M-wave was measurable, separate from the stimulus artifact (shown truncated) (see Fig. 4). The first, single M-wave was 9% M_{max} (1) and the H-reflex was relatively smaller measuring 1% M_{max} (2). From $time_1$ to $time_2$, the mean M-wave increased 41% from 7% M_{max} (1) to 10% M_{max}

(1) (P=0.01), and the mean H-reflex also increased from 0.3% M_{max} (0.3) to 1% M_{max} (0.2) (P=0.02). The corresponding torque increased 98% from 4% MVC (0.3) to 7% MVC (0.9) (P=0.001). Compared to responses during 20-Hz stimulation alone, after the 100-Hz burst all responses (torque and EMG) were larger (P=0.001).

Pooled results for CP nerve stimulation at 20-100-20 Hz at lower intensity are shown in Figure 5A (N=6). The first, single M-wave was 17% M_{max} (8) and the H-reflex was 5% M_{max} (10). From $time_1$ to $time_2$, the mean M-wave was unchanged from 12% M_{max} (5) to 14% M_{max} (6) (P=0.130), and the mean H-reflex was also unchanged from 0.8% M_{max} (0.8) to 2% M_{max} (2) (P=0.074). Nevertheless, the corresponding torque doubled from 2% MVC (1) to 4% MVC (2) (P=0.02). Although for the group EMG responses were not statistically different after the 100-Hz burst, individual differences were evident. These changes in mean values from before to after the 100-Hz burst are shown for individual subjects in Figure 5B. Extra torque in all 6 subjects was accompanied by increased mean M-waves and H-reflexes in 3 subjects. Across all subjects the magnitude of the mean H-reflex (range 0.3 to 4 % M_{max}) at $time_2$ was between 2- to 50-times smaller than the concurrent mean M-wave (range 6 to 24 % M_{max}). In the other 3 subjects, despite M-waves and H-reflexes not being significantly different after the 100-Hz burst, extra dorsiflexion of ~2% MVC was generated. However, the largest extra contractions, generating extra dorsiflexion of ~4% MVC, were accompanied with significant changes in the mean M-wave and the relatively smaller H-reflex (Fig 5B).

Higher intensity CP nerve stimulation

The effects of stimulating the CP nerve at higher intensity are shown in Figure 6 for a subject who exhibited large extra dorsiflexion. From $time_1$ to $time_2$, the mean M-wave increased from 49% M_{max} (7) to 55% M_{max} (5) (P=0.01), while the nearly absent mean H-reflex was slightly elevated from 0.3% M_{max} (0.1) to 0.5% M_{max} (0.1) (P=0.036). The corresponding torque doubled from 17% MVC (7) to 34% MVC (4) (P=0.001). In this case, however, the increased large M-waves were not significantly different from responses in the intensity-matched trial without the 100-Hz burst. Thus, in comparison to the trial without the burst, extra dorsiflexion of ~17% MVC appeared without sufficiently elevated EMG responses. Nonetheless, in another subjects extra torque was at least partially accounted by increased M-waves and the relatively smaller H-reflexes after the

100-Hz burst in comparison to responses without the burst (subject represented by dark square in Fig. 7B), in a similar manner as shown for a different subject in Figure 4.

Pooled results for 20-100-20-Hz stimulation of the CP nerve at higher intensity are shown in Figure 7A (N=7). The first M-wave was 45% M_{max} (18) and the H-reflex was 1% M_{max} (0.5). From $time_1$ to $time_2$, the mean M-wave was unchanged from 49% M_{max} (10) to 54% M_{max} (14) (P=0.065), while the relatively smaller mean H-reflex was also unchanged from 0.7% M_{max} (0.4) to 1% M_{max} (0.8) (P=0.128). The corresponding torque increased 55% from 13% MVC (7) to 20% MVC (9) (P=0.007). Although for the group the EMG mean responses were not statistically different after the 100-Hz burst, individual changes in mean values from before to after the 100-Hz burst are shown for subjects in Figure 7B. Extra dorsiflexion in all 7 subjects was accompanied by significantly increased mean M-waves in 3 subjects and significantly increased mean H-reflexes in 5 subjects. Across subjects the magnitude of mean H-reflex at $time_2$ (range 0.4 to 3% M_{max}) was between 20-100 times smaller than the concurrent mean M-wave (range 34 to 75 % M_{max}).

Discussion

The present purpose was to determine whether increased amplitude of H-reflexes contributed to extra torque generation by the contracting muscles following a 2-s 100-Hz stimulation burst. Since the amplitude of soleus H-reflexes increased substantially without consistent changes in the concurrently smaller M-waves, extra plantarflexion contractions appear to be predominantly reflexively driven. However, since the amplitude of TA H-reflexes was small and occasionally unchanged (5 of 13 cases), extra dorsiflexion contractions were most likely due to factors other than increased transmission through the H-reflex pathway.

Results of tibial nerve stimulation support earlier proposals that extra contractions involve activation of spinal motoneurons (Collins et al. 2001, 2002; Nickolls et al. 2004) because extra plantarflexion was accompanied by larger spinal reflex responses in the soleus muscle, overall free of peripheral changes (M-waves). On the contrary, results of CP nerve stimulation are indicative that extra dorsiflexion was due to other than reflexive contributions to: greater stimulation intensity was required, thus, necessitating a larger activation of motor axons (larger M-waves); sometimes the amplitude of the relatively larger M-waves increased in addition to changes in the smaller H-reflexes (6 of 13 cases,

including subject in Fig. 4), and extra contractions were present without sufficiently elevated M-waves or H-reflexes (5 of 13 cases, including subject in Fig. 6). These latter findings are suggestive of peripheral factors such as enhanced excitation and contraction of the TA muscle, or other central factors presently not investigated, such as asynchronous motoneuron discharge (Collins et al. 2001). The indications of peripheral contributions to dorsiflexion contrast previous findings of absent extra contractions during anesthetic block of the CP nerve proximal to the stimulation site (Collins et al. 2002) (see *Peripheral contributions to extra contractions).* Despite differences in the extent of reflexive and peripheral contributions, both such extra contractions may be advantageous over those evoked by conventional neuromuscular stimulation: due to greater reflexive recruitment of soleus motoneurons and enhanced excitability and contractibility of the TA muscle at a given intensity during stimulation of tibial and CP nerves, respectively (see *Implications*).

Reflexive contribution to extra contractions

Presently, enlarged spinal reflex responses independent of peripheral changes were most apparent during tibial nerve stimulation. Changes in spinal reflex responses during CP nerve stimulation, however, occurred mainly in conjunction with increased M-waves, thereby making interpretations about changes in transmission of TA H-reflex difficult to ascertain. Besides, the small TA H-reflexes probably did not contribute much to the overall torque and were subject to antidromic block due to greater excitation of motor axons, especially as higher stimulation intensity was required for extra dorsiflexion to develop. Thus, discussion of reflexive contribution to extra contractions is most relevant for outcomes of tibial nerve stimulation: i.e., for generating extra plantarflexion.

In the present study, an initial depression of H-reflexes (by 85% in the first half second) was in accord with the well known depression of H-reflexes during tetanic stimulation (Burke and Schiller 1976; Schindler-Ivens and Shields 2000; see INTRODUCTION). Considering the tendency for greater depression of spinal reflexes with increasing frequency of stimulation (Lloyd et al. 1957a,b; Schindler-Ivens and Shields 2000; Van Boxtel), as well as the reported complete depressions of H-reflexes evoked at 25 to 50 Hz (Burke and Schiller 1976; Lang and Vallbo 1967; Schindler-Ivens and Shields 2000), we presently expected a substantial depression of H-reflexes throughout the initial 20-Hz stimulation. However, we found that in some subjects a

rapid and pronounced recovery of soleus H-reflexes occurred during the initial 20-Hz stimulation (back to ~50% of the first H-reflex), and second, as hypothesized, in most subjects H-reflexes were generally larger after the 2-s 100-Hz stimulation burst. Thus, the 100-Hz burst was sufficient to further increase H-reflexes, but not necessary for some initial recovery to occur. These findings are in agreement with recent reports of partially recovered H-reflexes (up to ~20% of first H-reflex) during 50-Hz stimulation of tibial nerve afferents (Nozaki et al. 2003). Presently, the recovery was shown to be more substantial, independent of changes in M-waves, and in conjunction with extra muscle force (torque) generation.

Other studies that employed tetanic tibial nerve stimulation (1-ms pulses at 25 Hz to 100 Hz) demonstrated the emergence of asynchronous motoneuron discharge that was not time-locked to the stimulus, while H-reflexes remained depressed (Burke and Schiller 1976; Collins et al. 2001; Lang and Vallbo 1967). Such discrepancy in H-reflex behavior between these earlier studies and the present one may be explained by the size of the induced afferent volley, since stimulation was set mostly below motor threshold in the former (Burke and Schiller 1976; Lang and Vallbo 1967) and above motor threshold at the present. As a result, the present afferent volley may have been larger. In fact, the depression of the H-reflex during 2-Hz stimulation was shown to be less with larger initial responses (Van Boxtel 1986). Moreover, the ability to induce synchronized motoneuron discharge with high-frequency muscle vibration but not with low-intensity high-frequency electrical stimulation was in part explained by the size of induced afferent volleys, in addition to differences in afferent inputs and central transmission (Burke and Schiller 1976; Van Boxtel 1986). In animal studies, large monosynaptic reflexes are less readily abolished during ≤10-Hz stimulation than small reflexes; but with increasing frequencies (from 10 to 50 Hz) even large monosynaptic reflexes are progressively depressed (Lloyd and Wilson 1957). In human studies, even relatively large H-reflexes (20-50% M_{max}) undergo almost complete depression already during 10-Hz stimulation (Schindler-Ivens and Shields 2000). Among the present subjects in particular, the largest first H-reflex demonstrated least initial depression (i.e., most substantial recovery), whereas the smallest first H-reflex remained most depressed; yet across the subjects a clear relation between the size of the initial H-reflex and the extent of recovery was not obvious. Nevertheless, the extent of H-reflex recovery during tetanic stimulation may depend on the strength of the induced afferent volley.

Multiple mechanisms could contribute to increased amplitude of H-reflexes during 20-Hz stimulation. First, inadvertent or voluntary activation of motoneurons in response to the stimulation would increase H-reflexes, however, several pieces of evidence suggest this was not the case: subjects were instructed to relax during the stimulation, and when asked, reported to be relaxed; results were only considered if stimulation and contractions were comfortable and not painful; also in some cases H-reflexes recovered extensively even without the 100-Hz burst stimulation. Moreover, extra contractions similar to those in this study were previously shown in persons with clinically complete SCI (Nickolls et al. 2004), in which case sensation and descending drive would have been compromised. Second, reduction of presynaptic inhibition may underlie the presently transient changes in soleus H-reflexes since the level of presynaptic inhibition can be modified during the course of plantarflexion and by background EMG activity (Fumoto et al. 2002; Sato et al. 1999). Third, post-tetanic potentiation (PTP) of H-reflexes may have also been involved, possibly due to increased mobilization and release of neurotransmitter (Van Boxtel 1986); however, the presently used 2-s 100-Hz stimulation burst is shorter than stimulation periods typically required for PTP of H-reflexes to develop (tens of seconds to minutes) (Kitago et al. 2004; Van Boxtel 1986). Fourth, temporal summation of excitatory postsynaptic potentials could account for changes in H-reflexes, as shown for monosynaptic reflexes with stimulation above 60 Hz (Lloyd 1957a, b); however, the present 20-Hz stimulation was probably too low for temporal summation to be maintained. And lastly, activation of plateau potentials due to persistent inward currents in spinal motoneurons have been proposed to underlie the recovery of H-reflexes during stimulation (Nozaki et al. 2003), as well as to generate the drive for extra contractions (Collins et al. 2001, 2002; Nickolls et al. 2004), and could potentially amplify the given afferent input onto motoneurons (Lee and Heckman 2000; Powers and Binder 2000). However, previous notions that extra contractions are due solely to asynchronous motoneuron discharge sustained at their preferred rate by plateau potentials (Collins et al. 2001, 2002) were presently not supported since EMG activity was mostly synchronized to the stimulus (as in the H-reflex). Perhaps the motoneuron discharge, partially sustained by plateau potentials, is entrained to each stimulus pulse. At the present, in a few cases, some muscle activity was sustained after the stimulation ended (EMG not shown but made evident in slower declining torque in Fig. 2B), and so possibly some unmeasured asynchronous discharge may have occasionally contributed

during the stimulation. Yet unlike earlier reports in which sustained EMG activity was frequently seen (Collins et al. 2001; Nozaki et al. 2003), such activity was not as common in the present study.

Multiple mechanisms may be involved in circumventing the decreased release of neurotransmitter during tetanic stimulation (H-reflex depression; Hultborn et al. 1996). Examining changes in excitability at multiple sites of the H-reflex pathway may elucidate the involvement of each potential mechanism. An additional determinant of H-reflex amplitude is the stimulus pulse width. Presently 1-ms pulses were employed, which are optimal for eliciting the largest H-reflexes because afferents may be recruited preferentially to motor axons, due to differences in excitation of axons (Veale et al. 1973, Panizza et al. 1989). Indeed, wider pulses (1 vs. 0.05 ms) were concordantly demonstrated to evoke larger extra contractions of plantarflexors (but not of dorsiflexors) (Collins et al. 2002). In all likelihood, extra plantarflexion contractions are in large part due to greater reflexive recruitment of motor units of the soleus muscles. In fact an intact H-reflex pathway may be necessary, because extra contractions were shown to be absent during anesthetic block of the tibial nerve proximal to the site of stimulation at similar stimulation intensties (Collins et al. 2001, 2002).

Peripheral contributions to extra contractions

Extra plantarflexion contractions were explained mainly by a reflexive contribution unaccompanied by peripheral changes. On the contrary during extra dorsiflexion, TA H-reflexes were probably insufficient to generate the overall contraction torque, and so other factors need be considered. Extra dorsiflexion contractions usually required a greater activation of motor axons (larger M-waves), which would further block H-reflexes by antidromic collision especially at higher stimulation intensities. The aggregate data indicate that extra dorsiflexion contractions were not accompanied by changes in EMG responses. When examined on an individual basis, however, in some cases increased M-waves, in addition to the relatively smaller H-reflexes, accounted for extra dorsiflexion contractions. Thus, several peripheral mechanisms were probably involved to a different extent in each subject: firstly, increased peripheral excitation as reflected by M-waves and secondly, changes in excitation-contraction coupling, beyond those directly measurable by M-waves.

Firstly, extra dorsiflexion contractions were occasionally associated with enlarged TA M-waves after the 100-Hz burst (in 6 of 13 cases; see Fig. 4, 5B and 7B for examples). A larger M-wave would increase Ca^{2+} release, thereby affecting Ca^{2+} - dependent excitation-contraction coupling that would translate to increased force production (Klass et al. 2004; O'Leary et al. 1997). Comparable potentiation of M-waves has been shown to commence under the same stimulation parameters (2 s at 100 Hz) with nearly maximal activation of motor axons (McComas et al. 1994). In the same way, the presently potentiated M-waves likely reflected changes in excitation of the muscle fiber membrane (Cupido et al. 1996; McComas et al. 1994; O'Leary et al. 1997), because the stimulation intensity (current) was constant and at higher intensities an ample portion of motor axons was activated. Fluctuations in size of first few M-waves may be explained as mechanical artifact, consequent of muscle fibers shortening (McComas et al. 1994), whereas sustained M-wave potentiation is probably due to an enhancement of electrogenic Na^+-K^+ active-transport mechanism in the muscle fibers' membrane (Hicks and McComas 1989; Cupido et al. 1996; McComas et al. 1994). The paradoxical findings that presently potentiated M-waves likely contributed to extra dorsiflexion contractions in some cases and the earlier reported absence of extra contractions during proximal anesthetic block of the CP nerve (Collins et al. 2002), nevertheless, may be explained by a peripheral mechanism that depends on intact spinal innervations: indeed, norepinephrine released from intramuscular sympathetic nerve fibers is one probable stimulus to increase the electrogenic capacity of Na^+-K^+ active-transport mechanism, which would thereby amplify M-waves (Kuiack and McComas 1992; McComas et al. 1994).

Secondly, in some cases (5 of 13) extra dorsiflexion contractions were not accompanied by sufficiently increased M-waves or H-reflexes, especially when M-waves and contractions were large prior to the 100-Hz stimulation burst (>50% M_{max} and >10% MVC, respectively) (see Fig. 5B, 6A and 7B for examples). Such observations of potentiated contractions together with significantly larger evoked EMG responses are indicative of changes beyond the muscle membrane (O'Leary et al. 1997; Vandervoort and McComas 1983). In other studies, potentiated muscle twitches of ankle dorsiflexors without changes in M-waves immediately after tetanic stimulation (involving maximal M-waves at 100 Hz for 7 s) have been attributed to enhanced excitation-contraction coupling and/or myosin-actin interaction (O'Leary et al. 1997). The extent to which such

potentiation of muscle twitches contributed to extra dorsiflexion contractions under the present stimulation paradigm is not clear, since the magnitude of potentiation can be influenced by intensity, frequency and duration of stimulation (Brown and von Euler, 1938; Grange et al. 1993). Perhaps then at higher stimulation intensities, such extra dorsiflexion contractions would have had developed during previous investigations of nerve block of the CP nerve proximal to the stimulation site (see Collins et al. 2002).

The striking difference between the underlying mechanisms of the seemingly similar torque generation by dorsiflexors (TA) and plantarflexors (mainly soleus in the presently flexed knee position) may be ascribed to at least two main differences between these muscles. Firstly, the H-reflex is more predominant in soleus than in TA (Zehr 2002). Secondly, muscles with high proportions of fast-twitch fibers such as TA exhibit more prevalently both M-wave potentiation (McComas et al. 1994) and muscle twitch potentiation (Belanger et al. 1983; Brown and von Euler, 1938; O'Leary et al. 1997), whereas muscle with higher incidence of slow-twitch fibers such as soleus do not. In the case of extra contractions of plantarflexors, peripheral changes whether at muscle fibers' membrane or beyond were probably not involved because the soleus M-waves remained small and unchanged; and potentiation of plantarflexors is largely determined by the potentiating capacity of soleus, which is virtually absent (Vandervoort and McComas 1983).

Implications

For neuromuscular electrical stimulation (NMES) to achieve continuous muscle contractions, motor axons must be stimulated at 20 Hz and above (Popovic et al. 2001). From a clinical perspective, extra muscle contractions facilitated by intermittent high-frequency stimulation bursts may be advantageous over those evoked by conventional steady frequency NMES for several reasons (Collins et al. 2001). First, the reflexive contribution to extra plantarflexion contractions probably involves motor units otherwise not activated by stimulating motor axons alone, so this afferent stimulation may have application for reducing muscle atrophy. Second, since reflexive activation of spinal motoneurons may preferentially recruit fatigue-resistant motor units in a normal recruitment order (Awiszus and Feistner 1993; Buchthal and Schmalbrunch 1970; Henneman and Olson 1965), this may potentially be functionally important in reducing fatigue of evoked contractions (Collins et al. 2001). The presence of extra contractions in

other muscles remains to be investigated, and has additionally be demonstrated in wrist flexor muscles (Baldwin et al. 2004). The potential to generate reflexive extra contractions may necessitate substantial H-reflexes in the muscle (for listing of H-reflexes in muscles of the upper and lower limbs see Zehr 2002), and be limited by the stimulation intensity —large enough to overcome H-reflex depression and small enough to avoid antidromic collision (large M-waves). Moreover, by harnessing the larger than normal H-reflexes during tetanic stimulation in persons with chronic SCI (Schindler-Ivens and Shields 2000), such afferent stimulation may involve even greater spinal motoneurons otherwise not engaged in the contractions. Extra contractions have already been shown in persons with SCI (more frequently in plantarflexors than in dorsiflexors) without a clear association with hyper-reflexia and spontaneous spasms (Nickolls et al. 2004) and favorably, H-reflexes persist during repetitive stimulation in persons with SCI on occasions with diminished spasticity more so than with heightened spasticity (Ishikawa et al. 1966). For muscles in which both H-reflexes are less predominant and larger M-waves are required, as presently demonstrated in TA, extra contractions may still be advantageous over those conventionally evoked, by reducing fatigue hypothetically as a result of enhancements of the membrane excitation or excitation-contraction coupling; i.e., by maintaining the muscle fibers' electrogenic and contractile capacities (Cooper et al. 1988; Matsunaga et al. 1999), as well as enabling non-contracting fibers to participate once other motor units fatigue (McMomas et al. 1994).

Figures

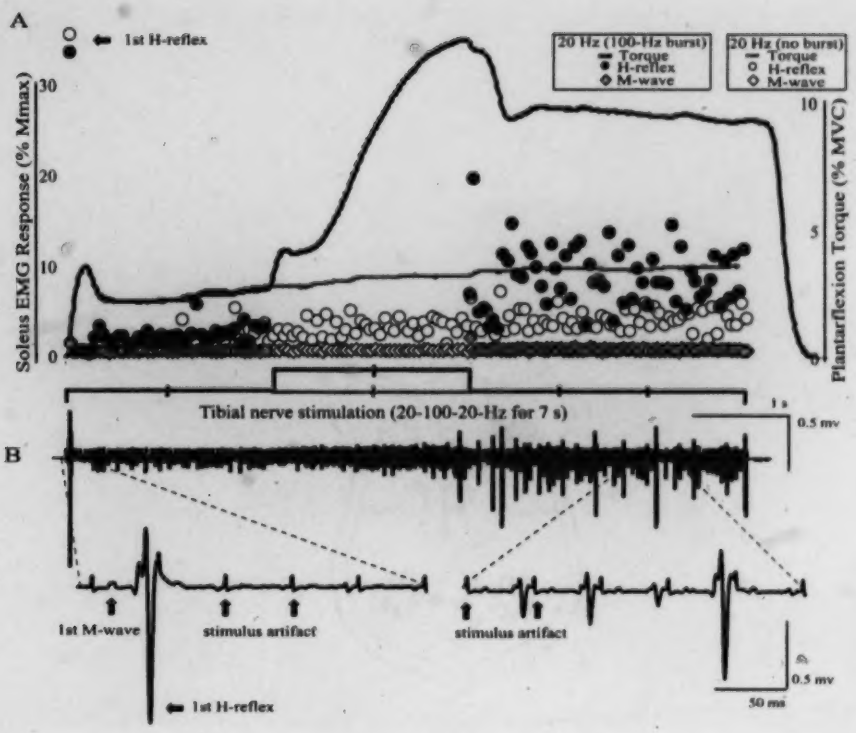

Figure 1. Plantarflexion and EMG responses evoked by tibial nerve stimulation in a single subject. A: normalized isometric torque generated by the 20-100-20-Hz stimulation, and normalized amplitude of each H-reflex and M-wave during 20-Hz stimulation before and after the 100-Hz burst. Also shown are torque, H-reflexes, and M-waves evoked by 20-Hz stimulation for 7 s (see legend for responses in trains with and without the 100-Hz burst). Data are averaged from 5 trains. Stimulation pattern with the 100-Hz burst (black line) and without the burst (grey line) are represented beneath the plot. B: Electromyograms from a single 20-100-20-Hz stimulation train, and beneath on an expanded time scale. Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 3B (grey triangle).

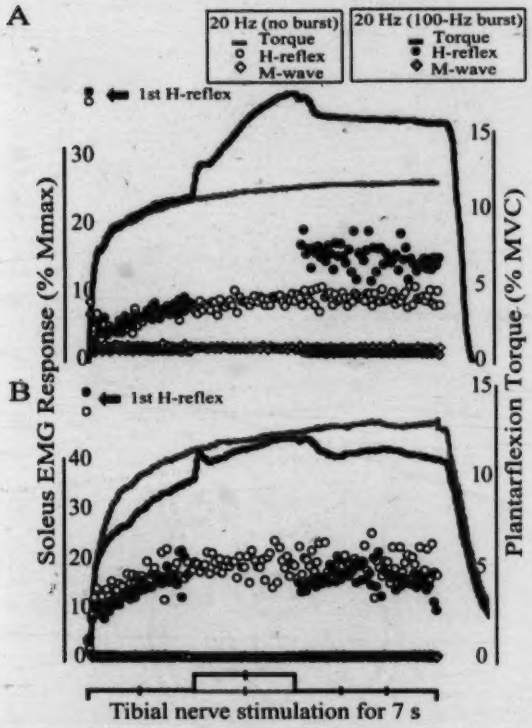

Figure 2. Effectiveness of the 100-Hz burst for increasing amplitude of H-reflexes and torque, compared to 20-Hz stimulation without the burst at matched stimulation intensity. A: pooled results (N=3) demonstrating increased H-reflexes after the 100-Hz burst. Mean responses, before and after the burst, are represented for each subject in Fig. 3B (dark square, dark diamond and grey triangle). Torque (grey line) from 20-Hz stimulation alone is truncated at 7 s because stimulation was 10 s in one subject. B: pooled results (N=3) demonstrating ineffectiveness of the 100-Hz burst at further increasing H-reflexes, compared to intensity-matched stimulation without the 100-Hz burst. Mean responses for each subject are represented in Fig. 3B (grey square, white triangle and white circle).

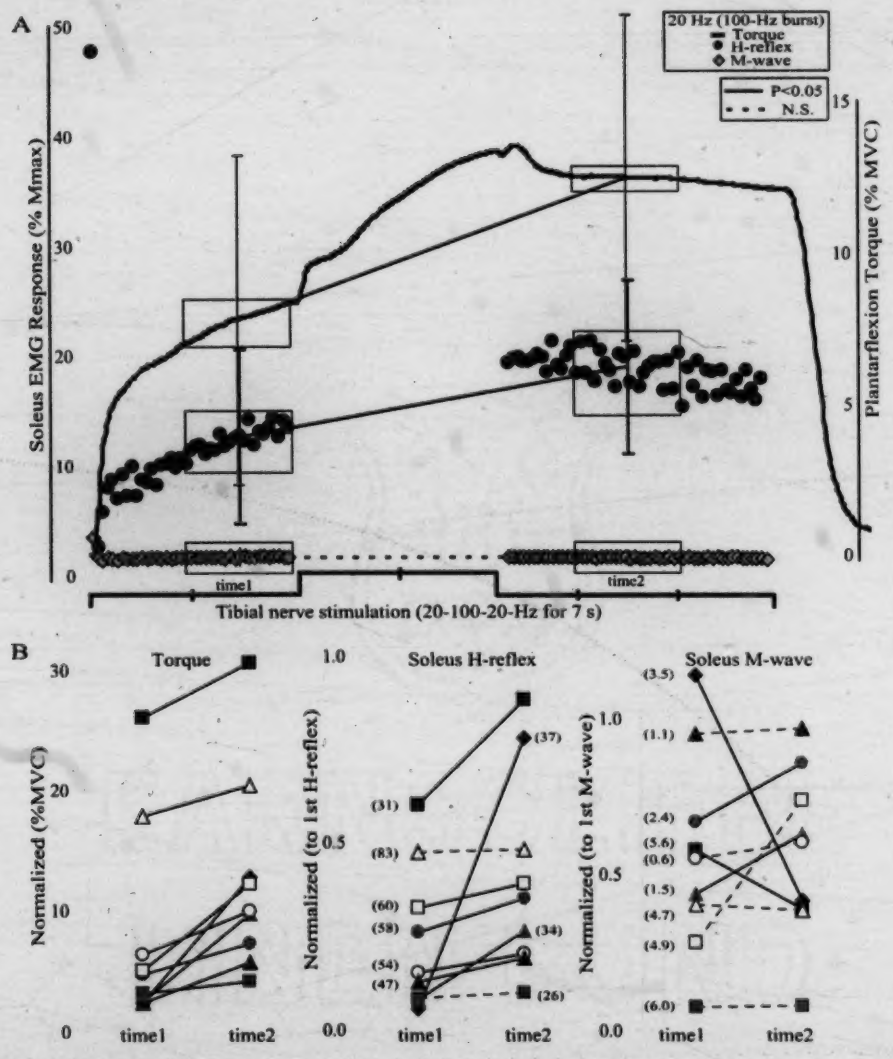

Figure 3. Group results of tibial nerve stimulation at 20-100-20-Hz for 7 s. A: pooled results of 9 subjects (vertical lines represent 95% confidence interval). B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the stimulation train; amplitude of this response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line, as well as N.S. changes by dashed lines.

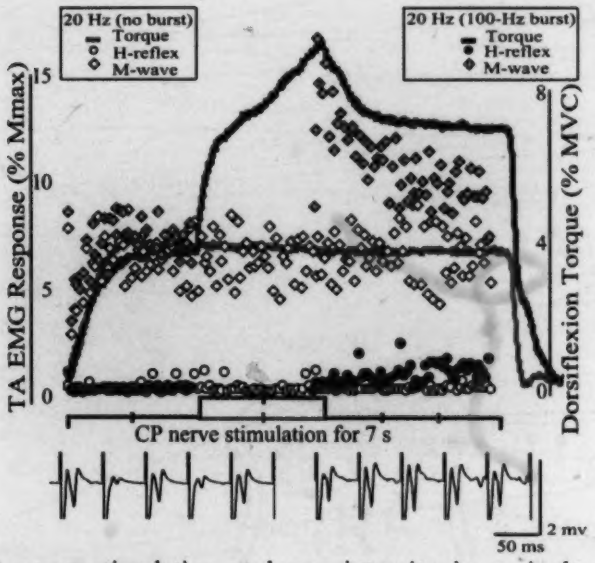

Figure 4. CP nerve stimulation at lower intensity in a single subject with extra dorsiflexion accompanied by increased TA M-waves and H-reflexes after the 100-Hz burst. Electromyograms are shown from a single train during 20-Hz stimulation, at onset (left) and after the 100-Hz burst (right). The amplitude of each M-wave was measurable apart from the stimulus artifact (truncated). Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 5B (grey triangle).

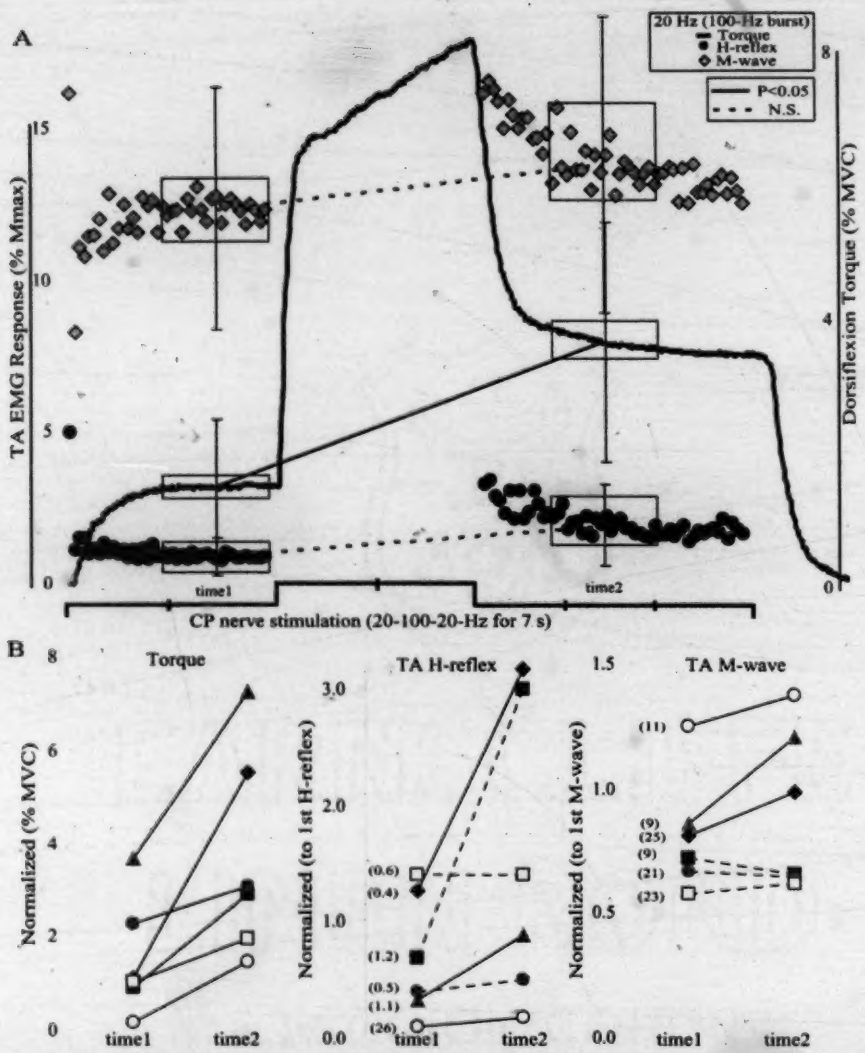

Figure 5. Group results of CP nerve stimulation at 20-100-20-Hz for 7 s at lower stimulation intensity (mean M-waves at $time_1$ were between 5-20% M_{max}). A: pooled results of 6 subjects (vertical lines represent 95% confidence interval. B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the train; amplitude of this response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line, as well as N.S. changes are denoted by dashed lines ($P=0.130$ and $P=0.074$ for pooled mean M-wave and H-reflex, respectively).

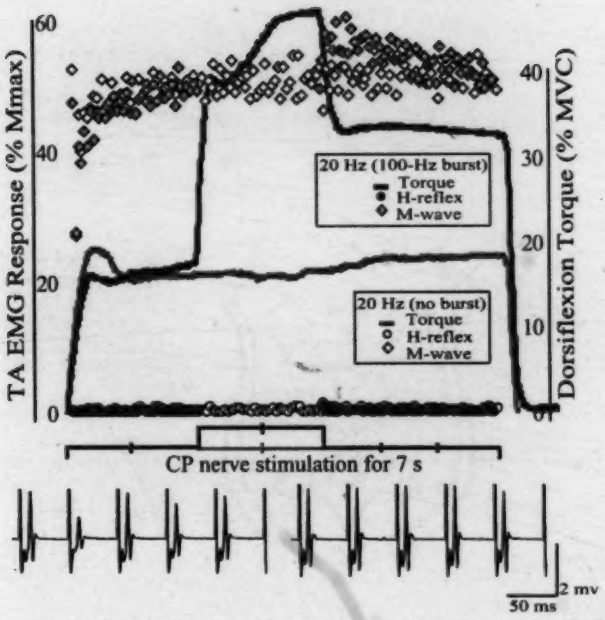

Figure 6. CP nerve stimulation at higher intensities in a single subject with extra dorsiflexion without sufficiently increased EMG responses after the 100-Hz burst. Extra torque was not associated with increased M-waves or H-reflexes, relative to EMG responses during 20-Hz stimulation alone. Mean responses before and after the 100-Hz burst are represented for this subject in Fig. 7B (white circle).

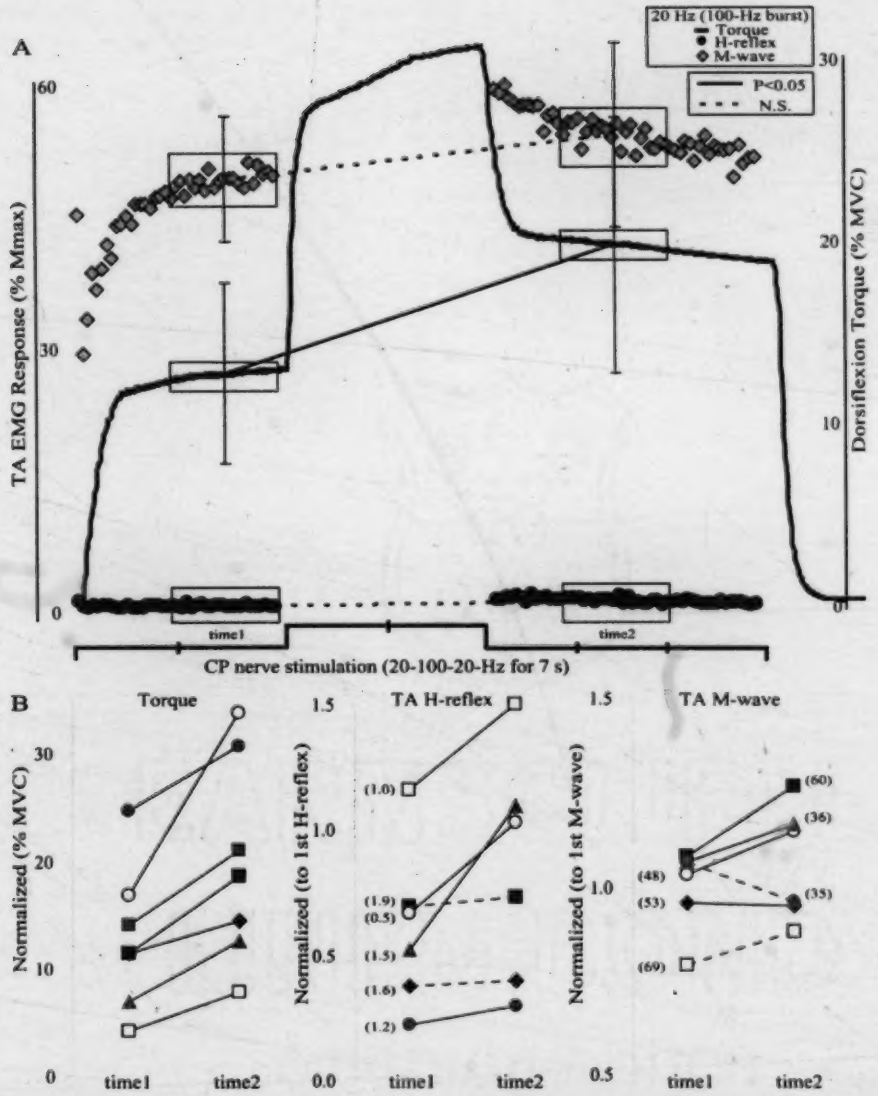

Figure 7. Group results of CP nerve stimulation at 20-100-20-Hz for 7 s at higher stimulation intensity (mean M-waves at $time_1$ were between 35-65% M_{max}). A: pooled results of 7 subjects (vertical lines represent 95% confidence interval). B: Mean responses before ($time_1$) and after the 100-Hz burst ($time_2$) for individual subjects. Individuals' mean H-reflex and M-wave are normalized to first response in the train; amplitude of the response is shown in parenthesis next to the symbol for each subject. For pooled and individual data, significant change ($P<0.05$) from $time_1$ to $time_2$ are denoted by solid line and N.S. changes are denoted by dashed lines ($P=0.065$ and $P=0.128$ for pooled mean M-wave and H-reflex, respectively). Note: for reasons of scale, mean EMG responses are not shown for one subject (grey square): at $time_1$ and $time_2$ the mean M-wave was 3 and 4 ($P=0.18$), respectively, normalized to the first M-wave (16% M_{max}), and the mean H-reflex was 0.3 and 2.7 ($P=0.005$), respectively, normalized to the first H-reflex (0.7% M_{max}).

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CHAPTER 3 – Conclusion and general discussion

At the onset of the study, enlarged H-reflexes were hypothesized to contribute to extra plantarflexion, since soleus H-reflexes can maximally activate the motoneuron pool and were know to rise from depression during tetanic stimulation. However, similar outcomes in the TA muscles were less expected because TA H-reflexes are less predominant and were unknown to exhibit such recovery. Differences notwithstanding, H-reflexes were evaluated in soleus and TA since apparently similar extra contractions forces were known to manifest in both muscles—presumable due to the same mechanisms. Undeniably, a greater central contribution—other than H-reflexes—was anticipated in the TA muscle. M-waves were certainly not expected to drastically change in either muscle because of previously reported absence of extra contractions during conditions of nerve block proximal to the stimulation site.

Afterthoughts on reflexive contributions to extra plantarflexion

The present findings of enlarged soleus H-reflexes, together with observations of mostly synchronized motoneuron discharge during the present experimental conditions (motor unit recording were not presented but appear in Lagerquist 2005a), support the conclusion that extra plantarflexion contractions are mostly reflexively driven. Somewhat surprising, was the finding that in some subjects H-reflexes and the resulting contractions were large prior to the 100-Hz burst. Technically such contractions were already extra to stimulating motor axons. Whether the present changes in H-reflex amplitude were determined largely by the amplitude or frequency of the induced afferent volley —by spatial summation or temporal summation of post-synaptic potentials— remains to be investigated.

Afterthoughts on other than reflexive contributions to extra dorsiflexion

Although TA H-reflexes were sometimes statistically significantly increased after the 100-Hz burst, their functional contribution was probably negligible relative to the M-waves, especially when large extra dorsiflexion contractions developed. Perhaps the most surprising finding was that in some cases the amplitude of M-waves increased after the 100-Hz burst, and sometimes even before it. Whether such potentiated M-waves depend on intact spinal innervations or purely peripheral mechanisms remains to be

investigated. Because extra dorsiflexion contractions were also presently observed in the absence of appropriate changes in M-waves or H-reflexes, at least two other possible factors were indicated. First, asynchronous motoneuron discharge may have been involved, which was not investigated, yet could not be inferred from the background EMG. At 20 Hz, the 50-ms inter-stimulus interval was practically too narrow to establish changes in background EMG –after all both an M-wave and an H-reflex, about 5-15 ms apart, were contained within the interval. Possibly some measures in which the H-reflex was negligible but statistically increased after the 100-Hz burst may have captured fluctuations in background EMG (due to asynchronous discharge) rather than an H-reflex per se. Second, in some other cases peripheral changes probably occurred beyond the muscle membrane (not captured in EMG signal). Most definitely, potentiated single muscle twitches of TA were evident after a 100-Hz burst in 2 subjects who exhibited extra contractions without sufficient changes in EMG responses (not presently shown): the post-tetanic twitches were larger, without changes in M-waves and H-reflexes, thus, clearly indicative of post-tetanic potentiation of muscle twitches. Such potentiation, however, was not observed in other subjects and was not formally investigated. Since stimulation intensity (as well as frequency and duration) determine the extent of TA muscle twitch potentiation, the influence of intensity in the present stimulation paradigm remains to be examined. Presently, it appears that when high stimulation intensity was used to evoke the largest M-waves, the resulting large extra contractions were least attributable to changes in evoked EMG responses. The employed "higher" intensity was larger than in the earlier studies of extra contractions, as reflected by the size of the initial muscle contractions as well as the "test" pulse (size of muscle twitch evoked with 5-pulses at 100 Hz; not shown by recorded). Nevertheless, when stimulation intensity was comparable to those used in earlier studies (test twitch of ~5% MVC), potentiated M-waves were still observed (see Fig. 4). Thus such peripheral changes are not expected to be unique to the present study and probably were involved in earlier studies of extra contractions of dorsiflexors.

Revisiting extra contractions during proximal nerve block conditions

Further experiments during nerve block conditions could address these indications of peripheral contributions to extra dorsiflexion contractions. Hypothetically, an absence of extra contractions without potentiated TA M-waves would indicate that both depend

on intact spinal innervations; whereas the persistence of potentiated M-waves in the absence of extra contractions would indicate that such recording of peripheral changes are superfluous —in which case larger M-waves do not translate to greater contraction force. Of course, the persistence of extra contractions with potentiated M-waves would challenge earlier conclusions of abolished extra contractions of dorsiflexors during nerve block conditions. Since changes beyond the muscle fiber membrane (twitch potentiation) have been indicated, it is conceivable that at higher-stimulation intensities extra contractions of the dorsiflexors will develop even under proximal nerve block conditions.

Reports of abolished extra contractions during nerve block conditions are not necessarily conclusive. These results are based on a total of 4 observed cases, and have not been consistently replicated. Attempts to demonstrate absent extra contraction using the same neuromuscular stimulation paradigm (over the muscle) have not been successful (Nielsen J., personal communication). Whether such a discrepancy is due to insufficient stimulation intensity during nerve block by Collins et al. (2001, 2002) or to the inability to induce a complete nerve block by others remains to be investigated.

Certainly the preset results of reflexively driven extra contractions of plantarflexors speak against the appearance of extra contractions during nerve block conditions. However, extra contractions evoked by stimulating the tibial nerve in the popliteal fossa compared to extra contractions evoked by stimulating over the belly may be constituted of differently evoked motor responses. The presently demonstrated extra contractions of plantarflexors are associated with enlarged soleus H-reflexes independent of changes in the smaller M-waves; however, apparently similar extra contractions during stimulation over the triceps surae muscles were shown to be associated with amplified soleus M-waves and increased but relatively smaller H-reflexes (Lagerquist et al. 2005b) —similar to the presently enlarged M-waves during CP nerve stimulation. Therefore, both types of stimulation types (nerve and over muscle belly) need be reinvestigated during nerve block conditions. One hypothesis may be that at lower stimulation intensities extra contractions will be absent during tibial nerve stimulation, because of blocked afferent volleys to evoke H-reflexes. However, at high enough intensity, at which point stimulation over the nerve and over the muscles will be similar —both involving large M-waves— extra plantarflexion contractions will appear even during nerve block conditions. Likewise extra dorsiflexor contractions are predicted to appear at higher stimulation intensities during nerve block conditions.

Experiments related to potentiated H-reflexes

The above experiments may distinguish the extent of peripheral and central contributions to extra contractions. Several other experiments may elucidate the site of amplification of soleus H-reflexes—whether pre-synaptic (thus due to greater transmission between the Ia terminal and the motoneuron) or post-synaptic (due to increased excitability of motoneurons). Rather than stimulating at 20 Hz (with an intermittent 100-Hz burst), lower frequency of stimulation would afford enough time to measure background EMG to determine changes in activity of the motoneural pool. Also, stimulating at 1-2 Hz would allow for comparison of classically potentiated H-reflexes after tetanic stimulation, attributed to increased neurotransmitter release. Moreover, an alternative drive to motoneurons can be used to determine the site of changes after the 100-Hz burst. For instance, larger motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) alternated with large H-reflex after a 100-Hz burst would be indicative of changes at post-synaptic sites; whereas unchanged MEPs alternated with larger H-reflexes would be indicative of changes at pre-synaptic sites. However caution must be applied in such interpretations since short-term tetanic afferent stimulation can change cortical excitability (Kido Thompson and Stein 2004).

Furthermore, stimulation at 10 Hz rather than 20 Hz may serve additional experimental purposes. For one, an unobstructed 50-ms window prior to the stimulus artifact would afford for the background EMG to be assessed. More importantly, the advantage of stimulating at 10 Hz (with an intermittent 100-Hz) would be that such outcomes can be compared to already quantified H-reflex depression in persons with and without SCI. Since limited data are available on the amplitude of H-reflexes during stimulation at 10-50 Hz, future experiments could also investigate H-reflex recovery during tetanic stimulation at different frequencies. Clinical neuromuscular stimulation typically employs such frequency of stimulation, but likely involves greater activation of motor axons. So the optimal stimulation intensity for maximal H-reflex recovery during tetanic stimulation could additionally be established. Large antidromic motor volleys would block H-reflexes, whereas small afferent volley could be insufficient for H-reflexes to re-emerge. A large afferent volley would probably synchronize motoneuron discharge (as in H-reflex), thus, if the objective is to evoke asynchronous motoneuron discharge then a smaller afferent volley may be of use after all.

In conclusion attempts to determine the mechanisms of extra contractions from analysis of apparently similar muscle force (torque) profiles fails to take into account differences in the extent of reflexive and peripheral drive. Therefore investigations of such contractions need be combined with measures of the underlying EMG responses.

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